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厦门大学

硕 士 学 位 论 文

雨生红球藻的培养及其虾青素的提取、稳定性和应用研究

Studies on *Haematococcus pluvialis* Culture Methods and Extraction, Stability and Application of Astaxanthin

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摘 要

虾青素是一种具有高经济价值的类胡萝卜素，鉴于其强大的抗氧化功能，在饲料、食品、保健品、化妆品及医药等行业均存在广泛应用和广阔的发展前景。作为所有已知的虾青素合成生物体中积累量最高的物种，雨生红球藻已成为近年来该领域的研究热点之一。

本文首先开展了雨生红球藻混合营养和异养培养方式下的最佳碳源及其浓度研究，结果表明，在混合营养和异养培养条件下，醋酸钠均是较丙二酸钠更适于雨生红球藻生长和虾青素积累的碳源，其最佳初始浓度分别为 1.0 $g·L^{-1}$ 和 2.0 $g·L^{-1}$。其细胞平均生长速率分别为 0.29 d^{-1} 和 0.136 d^{-1}；胁迫 7 d 后细胞干重和虾青素含量分别为 0.987±0.101$g·L^{-1}$，20.304±0.705 $mg·L^{-1}$ 和 0.763±0.051 $g·L^{-1}$，14.928±0.518 $mg·L^{-1}$，混合营养较对照组（光合自养）提高 25 %和 21 %，而异养培养则下降。因此结果表明以 1.0 $g·L^{-1}$ 醋酸钠为碳源的混合营养方式是适合雨生红球藻培养的最佳营养方式。

其次开展最佳营养方式结合半连续培养模式研究发现较一次培养，半连续培养模式下一定的更新率（20 %，30 %）能有效促进雨生红球藻的生长和虾青素的积累，其中 20 %更新率组的藻粉产率最高，达 1.32 $g·L^{-1}$；而 30 %更新率组的虾青素产率最高，达 22.94 $mg·L^{-1}$，但与 20 %更新率组差异不显著（p>0.05）。

此外利用光生物反应器培养雨生红球藻，能使雨生红球藻细胞获得快速生长，提高生物量，并缩短胁迫周期，但虾青素的产率并不高。

本文还开展了 CO_2 超临界萃取雨生红球藻中虾青素的工艺研究，在国内尚属首次。以虾青素提取率为指标，通过考察萃取压力、萃取温度、CO_2 萃取流速三因素对该指标的影响，应用了中心组合设计实验与响应面分析方法，建立了最佳工艺条件为：萃取压力 44.6 MPa，萃取温度 64.2 ℃，CO_2 流速 7.1 $L·h^{-1}$，萃取时间 3.5 h，在此条件下获得的虾青素提取率可达 1.028 %。

本研究还针对雨生红球藻来源的虾青素的稳定性开展研究发现：温度高于 40 ℃可影响色素的稳定性；光照可导致虾青素的降解，不同光照对虾青素稳定性影响作用的大小顺序为避光<室内光<室外光；虾青素对碱不稳定，碱浓度越

大，游离虾青素降解越严重，因此虾青素酯皂化过程中应选择合适的碱浓度和皂化时间，以减小碱对虾青素的降解作用；添加一定量（0.2 %）的 BHT 还原剂对虾青素有较好的保护作用，可使虾青素保存一周时间内含量基本没有变化。

同时探讨了雨生红球藻来源的虾青素对血鹦鹉观赏鱼的生长、着色及抗氧化能力的影响。结果表明与对照组相比，喂食添加虾青素饲料的鱼在生长、着色以及机体抗氧化能力均有显著提高。喂食添加虾青素饲料实验组鱼体增重 300 %，较对照组提高 50 %；鱼皮肤、鳞片中虾青素、类胡萝卜含量分别是实验前含量的 174 %，184 %和 207 %，256 %；鱼肌肉中的总抗氧化能力在实验过程中始终显著高于对照组。

关键词：虾青素；雨生红球藻；血鹦鹉；混合营养；异养培养；半连续培养；CO_2 超临界萃取；响应面法；稳定性；总抗氧化能力

Abstract

Astaxanthin, a naturally occurring carotenoid pigment, is a powerful biological antioxidant with high economic value for feedstuff, food, nutraceutical and cosmetics industries. Green alga *Haematococcus pluvialis*, which is the richest source of natural astaxanthin, has become the focus in recent years. In this thesis, culture methods of *Haematococcus pluvialis*, extraction of astaxanthin from *Haematococcus pluvialis*, and stability of astaxanthin were studied. Finally, bioactivity of astaxanthin was studied in an ornamental fish model.

Benefits of different carbon nutrition for *Haematococcus pluvialis* were investigated under mixotrophic and heterotrophic cultures. Results showed microalga grew better on acetate than on malonate in both culture modes. The optimum concentrations of acetate for its growth and accumulation of astaxanthin under mixotrophic and heterotrophic were 1.0 $g \cdot L^{-1}$ and 2.0 $g \cdot L^{-1}$, respectively. The specific growth rates of mixotrophic and heterotrophic were 0.29 d^{-1} and 0.136 d^{-1}. Cells dry weight and astaxanthin concentrations of mixotrophic or heterotrophic after 8-days' induction were 0.987±0.101$g \cdot L^{-1}$, 20.304±0.705 $mg \cdot L^{-1}$ or 0.763±0.051 $g \cdot L^{-1}$, 14.928±0.518 $mg \cdot L^{-1}$, respectively. Compared with phototrophic, the specific growth rate, cells dry weight and astaxanthin concentration of mixotrophic increased by 35 %, 25 %, 21 %. However, these parameters were decreased in heterotrophic. So mixotrophic with 1.0 $g \cdot L^{-1}$ acetate is a good choice for culturing *Haematococcus pluvialis*.

Semicontinuous cultures were compared with the batch culture under the former optimized trophic mode. Results showed the first stage of semicontinuous culture with daily renewal rates of 20 % and 30 % obtained 2.2 and 3.7 fold higher biomass than batch culture; in the second stage, the survival of alga under any renewal rates was remarkably higher than that of the control ($p<0.05$), while there were no significant differences between different renewal rates ($p>0.05$). The highest yield of astaxanthin

was 22.94 $mg \cdot L^{-1}$, obtained at a renewal rate of 30 %, which was not significant to that of 20 % renewal culture (p>0.05).

Besides, *Haematococcus pluvialis* cultured in the photobioreactor grew faster , enhanced the biomass and reduced stress cycle, however, the yield of astaxanthin was not higher.

A study of supercritical CO_2 extraction of astaxanthin from *Haematococcus pluvialis* was carried out. The effects of pressures, temperatures, CO_2 flow rates and time on extraction efficiency of astaxanthin were studied by the central composite experimental design principles and analyzed with Response Surface Method (RSM). The optimal conditions were determined: the highest extraction efficiency of astaxanthin was 1.028 % under the following conditions, the pressure of 44.6 MPa, the temperature of 64.2 ℃, the CO_2 flow rate of 7.1 $L \cdot h^{-1}$，extracting for 3.5 h.

Effects of temperatures, light, alkali concentrations and reducing agents on the stability of astaxanthin from *Haematococcus pluvialis* were studied. The results were as follows: the absorption value of astaxanthin decreased when temperatures were higher than 40℃; illumination and alkali concentrations can lead to the degradation of free astaxanthin; reducing agents can protect astaxanthin from oxidation, so astaxanthin contents did not changed in a week with 0.2 % BHT.

Effects of astaxanthin from *Haematococcus pluvialis* on the growth, pigmentation and antioxidant capacity of ornamental fish *Cichlasoma citrinellum x C. synspilum* were studied. Compared with control, the fish fed with astaxanthin-supplemented diet for 50 days gained over 50 % higher weight, 174 % and 184 % higher astaxanthin contents in the scales and skin, 207 % and 256 % higher total carotenoid contents in the scales and skin, and significantly higher total antioxidant capacity (TAC) in the muscle.

Key Words： Astaxanthin; *Haematococcus pluvialis*; *Cichlasoma citrinellum x C. synspilum*; Mixotrophic; Heterotrophic; Response Surface Method(RSM); Semicontinuous culture; Supercritical CO_2 Extraction ; Stability; TAC

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